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AMENDMENTS TO THE CLAIMS

Favorable reconsideration of this application, in light of the preceding

amendments and following remarks, is respectfully requested.

Listing of Claims

1. (Currently Amended) A method to determine a nucleotide sequence of a

target nucleic acid, comprising

a) contacting the target nucleic acid, or a fragment thereof, with a population of

capture oligonucleotide probes bound to a substrate at a series of spot locations, to

form probe-target duplex nucleic acids comprising single-stranded overhangs;

b) contacting the probe-target duplex nucleic acids with a population of Raman-

active oligonucleotide probes to allow binding of the Raman probes to the single-

stranded overhangs, wherein each Raman-active oligonucleotide probe generates a

distinct Raman signature, wherein at least one of the Raman-active oligonucleotide

probes comprises a positively charged Raman signal enhancer, the positively charged

Raman signal enhancer maintaining a positive charge capable of interacting with a

negatively charged species after binding with the probe-target complex:

c) detecting Raman-active oligonucleotide probes that bind the template nucleic

acid using Raman spectroscopy; and

d) identifying the location of the spot for each of the captured Raman-active

oligonucleotide probes, thereby determining a nucleotide sequence of the target nucleic

acid,

wherein at least one of the Raman-active oligonucleotides comprises a

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composite of organic-inorganic nanoparticles.

2. (Previously Presented) The method of claim 1, wherein each Raman-active

oligonucleotide probe intrinsically generates a detectable Raman signal.

3. (Previously Presented) The method of claim 2, wherein the at least one

Raman-active oligonucleotide is composed of less than 5 purine residues.

4. (Previously Presented) The method of claim 3, wherein the at least one

Raman-active oligonucleotide comprises no purine residues.

5. (Canceled)

6. (Original) The method of claim 1, wherein the determined nucleotide sequence

is a nucleotide occurrence at a target nucleotide position.

7. (Original) The method of claim 6, wherein the target position is a single

nucleotide polymorphism position.

8. (Original) The method of claim 1, wherein the determined nucleotide sequence

is a series of nucleotide occurrences at adjacent positions of a target segment.

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9. (Original) The method of claim 8, wherein the target segment is less than or

equal to the combined length of the capture oligonucleotide probe and the Raman-

active oligonucleotide probe.

10. (Original) The method of claim 8, wherein the target segment is less than or

equal to the length of the Raman-active oligonucleotide probe.

11. (Original) The method of claim 8, wherein the nucleotide sequence of the

entire target nucleic acid is determined by aligning detected target sequences.

12. (Original) The method of claim 1, further comprising ligating the capture

oligonucleotide probes to Raman-active oligonucleotide probes that bind to an adjacent

segment of the target nucleic acid.

13. (Original) The method of claim 1, wherein the target nucleic acid is isolated

from a biological source and contacted with the population of capture oligonucleotide

probes, without amplification.

14. (Original) The method of claim 13, wherein 1000 or less molecules of the

Raman-active oligonucleotide probe are detected.

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15. (Original) The method of claim 1, wherein the substrate is a biochip.

16. (Previously Presented) The method of claim 1, wherein the Raman-active

oligonucleotide probe is detected using surface enhanced Raman spectroscopy

(SERS).

17. (Original) The method of claim 1, wherein a first population of Raman-active

oligonucleotide probes are contacted with the probe-target duplex nucleic acids at a first

spot of a series of spots, and a second population of Raman-active oligonucleotide

probes are contacted with the probe-target duplex nucleic acids at a second spot of the

series of spots, wherein the first population of Raman-active oligonucleotide probes and

the second population of Raman-active oligonucleotide probes comprise at least one

different oligonucleotide probe.

18.-21. (Cancelled)

22. (Currently Amended) A method to determine a nucleotide occurrence at a

target nucleotide position of a template nucleic acid, comprising:

a) providing a labeled oligonucleotide probe that binds to the target

polynucleotide, wherein the labeled oligonucleotide probe comprises a first label and a

second label, the first label affecting the Raman spectra or fluorescent signal generated

by the second label based on the orientation of the first label to the second label,

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wherein the labeled oligonucleotide probe comprises a positively charged Raman signal

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enhancer, the positively charged Raman signal enhancer maintaining a positive charge

capable of interacting with a negatively charged species after binding with the probe-

target complex, wherein at least one of the first and second labels comprises a

composite of organic-inorganic nanoparticles;

b) contacting the labeled oligonucleotide probe with the target polynucleotide to

form a probe-target complex;

c) aggregating metal colloids or nanoparticles with the probe-target complex in

the presence of a mono-valent salt, wherein the metal colloids are pre-made;

d) applying an alternating current (AC) to the probe-target complex prior to

detection, wherein the applied AC enhances the difference in the affect of the first label

on the second label fluorescent signal or Raman spectra; and

e) detecting the fluorescent signal or Raman spectra generated by the second

label, wherein the nucleotide occurrence at the target nucleotide position affects the

orientation of the first label to the second label, thereby affecting the fluorescent signal

or Raman spectra generated by the second label and allowing determination of the

nucleotide occurrence at the target nucleotide position.

23. (Original) The method of claim 22, wherein a fluorescent signal is detected.

24. (Original) The method of claim 23, wherein the first label and the second

label are a FRET pair.

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25. (Original) The method of claim 24, wherein one label is TAMRA and another

label is ROX.

26. (Original) The method of claim 22, wherein a Raman spectra is detected.

27. (Original) The method of claim 26, further comprising comparing the detected

Raman spectra to a database of known spectra to identify the nucleotide occurrence at

the target nucleotide position of the target polynucleotide.

28. (Original) The method of claim 22, wherein the first label and the second

label are located about 3-6 nm apart on the labeled probe sequence.

29. (Original) The method of claim 22, wherein a series of nucleotide

occurrences for one or more target nucleotides are determined using a population of

labeled probes.

30. (Original) The method of claim 29, wherein probe-target complexes are

individually passed through an optical detector to read the fluorescent signal or Raman

spectra generated by the probe-target complexes.

31. (Original) The method of claim 29, wherein individual probe-target complexes

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are individually passed through an optical detector using a microelectromechanical

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system having a channel that is sufficiently narrow to allow only one probe-target

complex to pass.

32. (Previously Presented) The method of claim 22, wherein an alternating

current (AC) is applied at 10 mV to 100 mV, with an AC frequency of 1 Hz to 1 MHz.

33. (Currently Amended) A method for detecting a nucleic acid, comprising:

a) irradiating the nucleic acid with light, wherein the nucleic acid is covalently

attached to a positively charged Raman signal enhancer, wherein the positively charged

Raman signal enhancer comprises a primary amine Raman signal enhancer having an

alkyl chain of 1 to 25 carbon atoms and, the positively charged Raman signal enhancer

maintains a positive charge capable of interacting with a negatively charged species

after binding with the probe-target complex, wherein the positively charged Raman

signal enhancer comprises a composite of organic-inorganic nanoparticles;

and

b) detecting a Raman signal generated by the irradiated nucleic acid.

34. (Currently amended) The method of claim 33, wherein the nucleic acid

comprises less than 5 purine residues.

35. (Cancelled)

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36. (Original) The method of claim 33, wherein the nucleic acid consists of

pyrimidine residues.

37. (Original) The method of claim 1, wherein at least a portion of the single-

stranded overhangs is a constituent of the target nucleic acid.

38. (Previously Presented) The method of claim 1, wherein at least one of the

Raman-active oligonucleotide probes further comprises a Raman tag attached to at

least one of the Raman-active oligonucleotide probes.

39.-40. (Cancelled)

41. (Previously Presented) The method of claim 1, further comprising

aggregating pre-made metallic colloid or aggregate of nanoparticles with said at least

one of the Raman active oligonucleotide probes comprising the positively charged

Raman signal enhancer.

42. (Previously Presented) The method of claim 33, further comprising

aggregating pre-made metallic colloid or aggregate of nanoparticles with said nucleic

acid covalently attached to a positively charged Raman signal enhancer.

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43. (Previously Presented) The method of claim 1, wherein the positively

charged Raman signal enhancer comprises a primary amine Raman signal enhancer

having an alkyl chain of from 1 to 25 carbon atoms.

44. (Previously Presented) The method of claim 41, wherein said aggregating is

in the presence of a mono-valent salt.

45. (Previously Presented) The method of claim 1, wherein the positive charge

on the positively charged Raman signal enhancer is carried by a heteroatom, the hetero

atom excluding N, O, and S.